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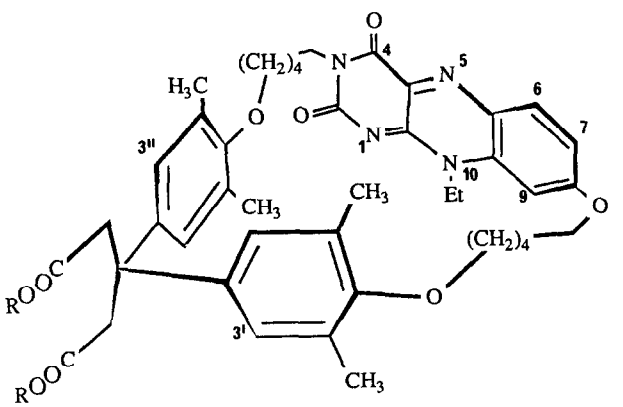
REDOX-DEPENDENT COMPLEXATION ABILITY OF FLAVIN-HOSTS IN AQUEOUS SOLUTION

Eileen Seward and François Diederich*
Department of Chemistry and Biochemistry, University of California
Los Angeles, California 90024, USA.

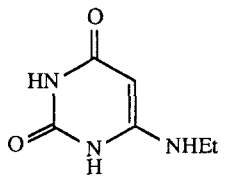
Abstract: The synthesis of the novel macrocyclic host **1** incorporating an isoalloxazine moiety as model for active sites of flavoenzymes is described. The complexation between oxidized and reduced flavin-host and aromatic guests is analyzed in aqueous solution.

Flavin coenzymes[flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD)] are known to be tightly bound or, in some cases, even covalently attached to the active sites of flavoenzymes.[1]The isoalloxazine unit in the oxidized form of the coenzyme is planar whereas the dihydroisoalloxazine unit in the $2e^-$-reduced form takes a butterfly shape by bending with an angle of $\approx 30^o$ around the two nitrogens N-5 and N-10 of the central ring in the tricyclic system. Depending on their function, the enzymes can either stabilize the planar oxidized or the bent reduced form and thus alter considerably the redox potential of the flavin. Controversial discussions have focused on the importance of electron donor-acceptor interactions in biotic and abiotic systems between electron-deficient isoalloxazines and aromatic donor molecules as well as between electron-rich dihydroisoalloxazines and aromatic acceptors.[2] In this paper, we present the synthesis and a preliminary analysis of the complexation behaviour of the macrocyclic host **1** which incorporates an isoalloxazine unit and mimics the active sites of flavoenzymes.[3] Host **1** was designed to study how the difference in geometry between the oxidized and the reduced form of the isoalloxazine unit affects the complexing ability of a molecular binding site[4] and to which extent electron donor-acceptor interactions stabilize complexes of aromatic guests in aqueous solution.[5]



1 R = H
2 R = C_2H_5

In the synthesis of **1**, conversion of 6-chlorouracil with ethylamine in a sealed tube at 120°C afforded 6-(*N*-ethyl)aminouracil (**3**, 70%), which reacted with an excess of *p* -nitrosoanisole in acetic acid/acetic anhydride to give the isoalloxazine **4** (60%).[6,7] Demethylation with boron tribromide in 1,2-dichloroethane provided the phenol **5** (95%)[6], which was alkylated with 1,5-dichloropentane (DMF/Cs_2CO_3) to yield the dichloride **6**[6] in 69% yield. The diphenol **7** [6], a new versatile building block for water-soluble macrocyclic hosts, was obtained in 75% from diethyl 1,3-acetonedicarboxylate and 2,6-dimethylphenol in the presence of 80% sulfuric acid. Cyclization of the dichloride **6** with the diphenol **7** (DMF/Cs_2CO_3) afforded the bright yellow macrocycle **2** [6], mp 252 - 253°C in 12 % yield. Hydrolysis of the diester **2** to the yellow target host **1**[6], mp 293-294° C was accomplished with stoichiometric amounts of methanesulfonic acid in formic acid (80°C, 12h; 90%).



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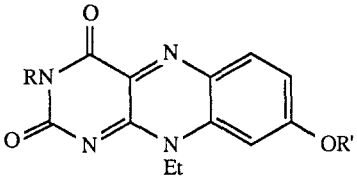
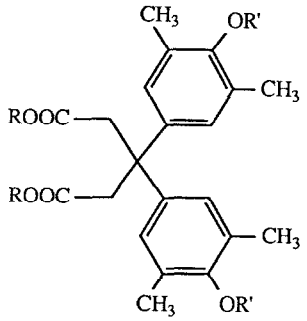
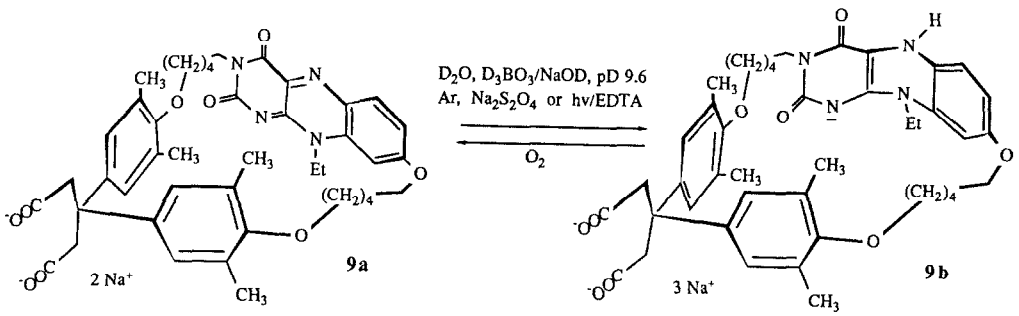


4 R = H, R' = CH_3
5 R = R' = H
6 R = R' = $(CH_2)_5$-Cl



7 R = C_2H_5, R' = H
8 R = H, R' = CH_3

The oxidized flavin-host **1** is readily soluble in alkaline deuterated borate buffer (D_2O, D_3BO_3/NaOD; pD = 10.4). Reduction of the bright yellow solution of the dicarboxylate **9a** under argon with sodium dithionite or photochemically using a 220 W daylight lamp in the presence of ethylenediaminetetraacetate (EDTA) yielded quantitatively the almost colorless solution of the reduced trianionic[8] host **9b**. The quantitative character of the reduction is fully supported by ^1H NMR and electronic absorption spectroscopy (Figure 1). In addition, the bright greenish fluorescence (λ_{exc}= 450 nm, λ_{em}= 492 nm) of



9a **9b**

9a disappeared completely upon reduction. By introduction of oxygen into the solution of **9b**, the oxidized flavin-host **9a** is regenerated quantitatively. The redox cycles can be repeated numerous times without affecting the molecular structure of the host. CPK model examinations suggest that the shown conformation of free **9b** with the dihydroisoalloxazine bending outward and opening the cavity is favored over the conformation with the isoalloxazine bending inward and closing a possible binding site. This is experimentally supported by the downfield shifts, that the methyl group protons (-0.27 ppm) and the aromatic protons (- 0.23 ppm) of the diphenylmethane unit encounter upon reduction of **9a** to **9b**.

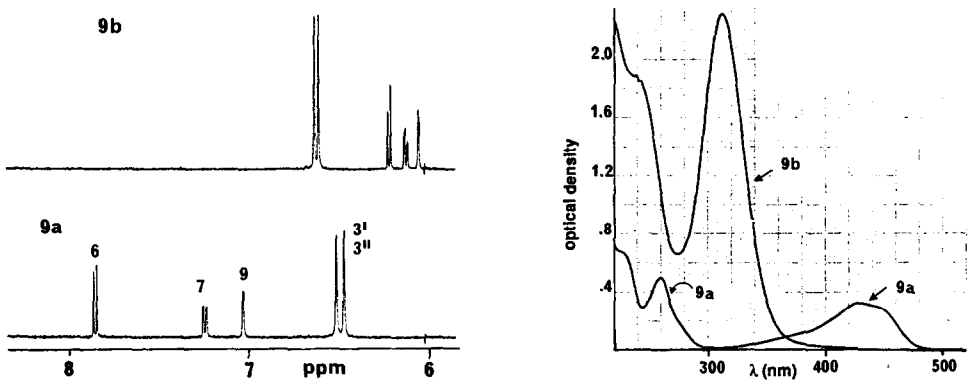


Figure 1: ^{1}H NMR spectra (500 MHz, T = 293K, c = $2x10^{-3}$ mol·L^{-1} in borate buffer, pD = 10.4, HDO-peak as int. standard, only aromatic region shown) and electronic absorption spectra (d = 0.2 cm, c = 7.5×10^{-5} mol·L^{-1} in the borate buffer) of **9a** and **9b**. The reduced solutions under argon contain [$Na_2S_2O_4$] = 0.2 mol·L^{-1}.

^{1}H NMR (500 MHz, T = 293K) host-guest complexation analysis with **9a** and **9b** and, for comparison, with compounds **4** and **8** was undertaken in deuterated borate buffer (pD = 10.4) in concentration ranges below c = 5×10^{-3} mol·L^{-1}, an upper limit defined by the aggregation of the macrocycles. Solutions of **9b** and of the reduced form of **8** were prepared under argon with $Na_2S_2O_4$ (c= 0.2 mol·L^{-1}) .The ^{1}H NMR spectra show complete absence of binding interaction between the non-macrocyclic comparison compounds **4** or **8** and aromatic guests. Both macrocycles **9a** and **9b**, however, act as hosts and form 1:1 complexes of significantly different geometry with 2,6-disubstituted naphthalenes. In the cavity of the reduced host **9b**, the guests are located preferentially in the plane defined by the central carbon atom of the diphenylmethane unit and the nitrogen atoms N-5 and N-10 of the dihydroisoalloxazine unit. This geometry of complex is supported by the position-dependent strong upfield shifts of the guest protons, the upfield shifts of the aliphatic bridges of **9b**, and the downfield shifts of all aromatic protons and the protons CH_2COO^- of the host. CPK model examinations suggest that guests can only fit into the cavity of the oxidized host **9a** if they are oriented almost cofacially to the isoalloxazine moiety. In support of this geometry, the calculated upfield complexation shifts at saturation binding ($\Delta\delta_{sat\ calc}$)[9] of the protons of the guests are considerably smaller in complexes of **9a** than in complexes of **9b**. Also, the isoalloxazine protons are shifted upfield.

Differences in the stability of the complexes of **9a** and **9b** can result from differences in the charge and geometry of the binding sites as well as from the electronic complementarity between host and guest. Complexes of comparable stability form between both hosts and 2,6-donor-donor or donor-acceptor substituted naphthalenes. The association constants of the **9a**·6-hydroxy-2-naphthonitrile complex (K_a = 163 L·mol^{-1}) and of the **9b**·6-hydroxy-2-naphthonitrile complex (K_a = 245 L·mol^{-1}) illustrate the observed magnitude of binding.[9] A surprisingly large difference is observed in the complexation of 2,6-acceptor-acceptor substituted naphthalene guests. The ^{1}H NMR titration in borate buffer/methanol-d_4 (1:1)[10] showed almost complete absence of complexation between host **9a** and 2,6-dicyanonaphthalene since no upfield complexation shifts ($\Delta\delta_{obs}$) of the guest signals were observed at [**9a**]= 3 x 10^{-3}mol·L^{-1} and [guest] = 2 x 10^{-4}mol·L^{-1} [$\Delta\delta_{obs}$: 0.01 (1-H), 0.00 (3-H), 0.01 (4-H)]. Under the same conditions with **9b**, the observed complexation shifts are: $\Delta\delta_{obs}$: 0.24 (1-H), 0.08 (3-H), 0.15 (4-H). The large difference in the binding of electron-deficient aromatic guests still has to be expressed in terms of quantitative data. We explain the difference by favorable electronic complementarity between the electron-rich host **9b** and these guests and by electrostatic repulsion, including unfavorable solvation patterns, between the isoalloxazine moiety of **9a** and the guests. Electronic absorption spectra taken from solutions of complexes formed between **9a/9b** and ten different aromatic guests, provided no evidence for charge-transfer interactions.

Extensions of the studies with **9a/9b** now focus on additional quantitative binding data, especially involving biological substrates, on the determination of structures of **9a/9b** by X-ray analysis and computer modelling, and on the catalysis of redox processes[11] in supramolecular complexes.

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9) Association constants and complexation shifts at saturation binding $\Delta\delta_{sat\ calc}$ were determined from non-linear least square fitting of NMR titration curves ([H_0] = 4 x 10^{-4}- 4 x 10^{-3} mol·L^{-1} and [G_0] = 5 x 10^{-4} mol·L^{-1}). Examples (ppm): **9a**·6-hydroxy-2-naphthonitrile: 1-H_G: $\Delta\delta_{max\ obs}$= 0.45; $\Delta\delta_{sat\ calc}$ = 1.15. **9b**·6-hydroxy-2-naphthonitrile: 1-H_G: $\Delta\delta_{max\ obs}$ = 1.31; $\Delta\delta_{sat\ calc}$= 2.74.
10) Very low solubility of electron-deficient guests like 2,6-dicyano- and 2,6-dinitronaphthalene has prevented studies in pure aqueous solution. The stability of complexes in methanolic aqueous solution is considerably reduced but the sequence of binding strength, observed within a wide range of guests, is identical in both solutions.
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